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Carcass Characteristic, Caecal Metabolites and Dropping Quality in Broiler Chickens Fed Diets Containing a Humic Substances

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ABSTRACT— *The objective of the study was to determine the effect of humic substances added to diets on carcass characteristics, weight of visceral organs, fermentation process in the caecum and excretion of dry matter and crude protein in broiler chicks. One hundred one day old broiler chicks were assigned in two equal groups. Birds of the experimental group were fed with diets supplemented with humic substances (in amount: 5 g.kg-1 during the first two weeks and 7 g.kg-1 from the 3rd to the 6th week). No significant effect of humic substances supplementation was observed on carcass characteristics (carcass weight, carcass yield, abdominal fat pad) as well as relative weight of the liver, the heart, the spleen and the bursa of Fabricius. On the other hand, in the experimental group was found significantly higher relative weight of the pancreas (P < 0.05). Also, concentration of short-chain fatty acids in the caecum content was not significantly affected, but tested substance lowered the pH value in caecum (P < 0.05). Significantly lover content of crude protein (P < 0.05) and also significantly higher concentration of dry matter (P < 0.01) in the droppings of chicks was found in experimental group than in the control group. It was concluded that the addition of humic substances to the diets can contribute to reducing the burden on the environment from poultry farms.*

Keywords— carcass yield, short-chain fatty acids, excretion, poultry

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1. INTRODUCTION

Humic substances are products of chemical and biological degradation of dead plant and animal tissues in soil, drinking water and sea water, which bind metals, organic and inorganic compounds including toxic compounds by forming chelates. They occur naturally in peat, brown coal, lignite and some other materials. Humic acids, fulvic acids and humins are considered the main fractions of these substances. In addition to these substances, they also contain ulmic acids and trace amounts of minerals such as iron (in the highest amount), manganese, copper and zinc [1; 2].

Additives based on humic acids are currently used primarily in plant production to increase soil fertility and plant biomass production [3]. In medicine, they are used to treat various diseases (heavy metal poisoning, diseases of the digestive tract, etc.) [4]. Recently, considerable attention has also been paid to the possibility of their use in livestock production. Many authors in their studies noted improvement in poultry production parameters and nutrient utilization after adding humic substances to feed [5 - 8] or to water [9; 10]. Since humic substances promote growth, they are a suitable alternative to antibiotic growth stimulators. Moreover, they may have immunostimulatory, anti-inflammatory and antiviral effects [11].

One of the most serious environmental problems in intensive poultry farming is high nitrogen excretion. This nitrogen is in the litter converted through microbial fermentation to volatile ammonia, whose higher concentration in the air of stud areas negatively affects health and performance of animals as well as health of farm staff [12]. It was found that in the poultry-raising, 18 per cent of fed nitrogen is released to the atmosphere in the form of ammonia [13]. Also it was found that by applying humates to the floor of stables, the content of free ammonia in the air can be reduced. The ammonia-saturated sorbent can then be used as a nitrogen-rich fertilizer [14; 15].

The objective of this experiment was to study the influence of humic substances on some carcass characteristics, processes of digestive tract and excretion in broiler chicks.

2. MATERIALS AND METHODS

2.1 Animals and diets

The experiment was carried out in accredited stables of the Department of animal nutrition and husbandry at the University of Veterinary Medicine and Pharmacy in Košice in compliance with the EU regulations concerning the protection of experimental animals. The experiment was carried out with the consent of the institutional Animal Care and University Ethics Committee.

In this research, one hundred unsexed one day old broiler chicks (Ross 308) obtained from a commercial supplier were used. Chicks were weighed, randomly divided into two equal groups (control and experimental group; 50 chicks per group) and housed on deep bedding in agreement with the technological instruction for Ross 308 chicks, with controlled light, temperature, animal hygiene and feeding regime. The lighting schedule was 24 h of light per day.

Complete mixtures in mash form (according to the stages of growth) and drinking water were offered to birds *ad libitum.* Diets were based on corn, soybean meal and wheat (Table 1). The experimental group diets were supplemented with humic substances (oxyhumolite - total humic acids 68 %, free humic acids 48 %, minerals 18 %; locality Dudar, Hungary) at different amounts: 0.5 % during first two weeks and 0.7 % from week 3 to 6. The introduction of the humic substances supplement into the diets was realized at the expense of wheat in the experimental group. No antibiotic growth promoters or anticoccidial drugs were used in the diets.

Table 1: Composition of diets

	Starter diet (1st –2nd week)		Grower diet (3rd – 5th week)		Finisher diet (6th week)	
	Control group	**Experimental group**	**Control group**	**Experimental group**	**Control group**	**Experimental group**
Ingredients (%)						
Corn	43.5	43.5	50.0	50.0	50.0	50.0
Soybean meal	36.0	36.0	33.0	33.0	31.0	31.0
Wheat	12.1	11.6	9.0	8.3	10.4	9.7
Vegetable oil	4.0	4.0	4.0	4.0	5.0	5.0
Limestone	2.0	2.0	1.6	1.6	1.5	1.5
Vitamin-mineral premix	2.0[1]	2.0[1]	2.0[2]	2.0[2]	2.0[3]	2.0[3]
Lysine	0.4	0.4	0.4	0.4	0.1	0.1
Humic substances	–	0.5	–	0.7	–	0.7
Chemical composition (%)						
Dry matter	89.69	89.56	90.02	89.80	89.39	89.66
Crude protein	24.99	25.12	23.05	22.56	21.87	21.46
Ether extract	7.01	6.95	7.19	6.97	8.03	8.18
Crude fiber	3.67	3.71	4.43	4.23	4.26	3.63
Crude ash	8.23	8.17	6.69	6.89	6.60	7.08
ME ($MJ.kg^{-1}$)	13.30	13.30	13.30	13.30	13.50	13.50

[1]Vitamin and Mineral premix (per kg): Ca 95 g, P 135 g, Na 75 g, Mg 5 g, DL-methionine 80 g, vit.A 600,000 IU, D3 135,000 IU, E 900 mg, K3 150 mg, panthotenic acid 600 mg, niacin 4000 mg, cholin chloride 20,000 mg, B6 150 mg, B12 900 μg, biotin 3000 μg, folic acid 76,000 μg, vit. C 2000 mg, Fe 1500 mg, Cu 500 mg, Zn 3000 mg, Mn 5000 mg, I 25 mg, Se 23 mg, Co 10 mg;
[2]Vitamin and Mineral premix (per kg): Ca 100 g, P 135 g, Na 75 g, Mg 5 g, DL-methionine 80 g, vit. A 425,000 IU, D3 84,000 IU, E 900 mg, K3 100 mg, pantotenic acid 420 mg, niacin 3400 mg, cholin chloride 14,200 mg, B6 100 mg, B12 640 μg, biotin 2150 μg, folic acid 54,500 μg, vit.C 1400 mg, Fe 1500 mg, Cu 500 mg, Zn 3000 mg, Mn 5000 mg, I 25 mg, Se 23 mg, Co 10 mg;
[3]Vitamin and Mineral premix (per kg): Ca 110 g, P 145 g, Na 75 g, Mg 9 g, DL-methionine 55 g, vit. A 370,000 IU, D3 135,000 IU, E 900 mg, K3 95 mg, panthotenic acid 370 mg, niacin 3880 mg, cholin chloride 14,000 mg, B6 95 mg, B12 560 μg, biotin 1850 μg , folic acid 47,000 μg, vit.C 1240 mg, Fe 1500 mg, Cu 500 mg, Zn 3000 mg, Mn 5000 mg, I 25 mg, Se 23 mg, Co 10 mg.

2.2 Sampling and measurements

At the age of 35 days, eight birds from each group were weighed and slaughtered by cervical dislocation to determine relative weight of organs (liver, heart, spleen, bursa of Fabricius and pancreas), and to determine pH and concentration of short-chain fatty acids in caecum contents. Relative organ weight was calculated as a percentage of live body weight. The pH value of caecum contents was determined by pH-meter (Consort C830, Belgium). The concentration of fatty acids (acetic acid, propionic acid, butyric acid, lactic acid) was analysed by isotachophoresis using a two-capillary isotachophoretic analyser (EA100, VILLA LABECO, Slovak Republic).

At the end of the trial (42nd day), the birds were left for 10 - 12 h without feed, weighed and slaughtered by cervical dislocation, processed by decapitation, neck, feathers and feet removal and evisceration. Twenty birds per group (ten from each sex) were used for evaluation of carcass yield and abdominal fat pad.

The faeces were collected thrice a day every day during the fifth week. The collection of faeces from random chickens in each group was made on clean solid base immediately after excretion to eliminate any contamination with raw feed or feathers. Composite samples from each group in appropriate amounts were frozen and kept at −18°C until analysis for dry matter and crude protein content.

Dry matter, crude protein, ether extract, crude fiber, crude ash in the diets and dry matter and crude protein in the faeces were analysed according to Association of Official Analytical Chemists [16].

2.3 Statistical analysis

Statistical evaluation of the effects of humic substances on monitored parameters was done by unpaired t-test with the statistical software GraphPad Prism 8.0. For all statistical calculations, the significance was considered as a value of $P < 0.05$.

3. RESULTS

The body weight before slaughter, the carcass weight, the carcass yield and the percentage of abdominal fat pad from the carcass weight were not statistically significantly affected by the addition of humic substances. In the experimental group (both hens and roosters) the values were comparable to the control group (Table 2).

No significant differences were also found in the relative weight of liver, heart, spleen and bursa of Fabricius (Table 3). The relative weight of the pancreas was significantly higher in the experimental group than in the control group ($P < 0.05$).

Table 2: Effect of humic substances on carcass characteristics

	Control group	Experimental group	P-value
Body weight before slaughter (g)			
Female	2420 ± 38.79	2395 ± 50.37	0.7045
Male	2867 ± 52.64	2823 ± 58.18	0.5861
Total	2643 ± 60.35	2609 ± 61.75	0.6959
Carcass weight (g)			
Female	1799 ± 38.16	1772 ± 43.67	0.6532
Male	2114 ± 41.28	2069 ± 43.18	0.4561
Total	1956 ± 45.37	1920 ± 45.29	0.5777
Carcass yield (%)			
Female	74.02 ± 0.56	73.94 ± 0.40	0.9112
Male	73.74 ± 0.22	73.28 ± 0.38	0.3074
Total	73.87 ± 0.28	73.61 ± 0.28	0.5158
Abdominal fat pad (% carcass weight)			
Female	2.03 ± 0.23	1.83 ± 0.12	0.4345
Male	1.38 ± 0.15	1.75 ± 0.12	0.0590
Total	1.72 ± 0.15	1.79 ± 0.08	0.6825

Data are presented as means ± standard error of means.

Table 3: Effect of humic substances on relative weight of visceral organs (% of live body weight)

	Control group	Experimental group	P-value
Liver	2.015 ± 0.129	1.942 ± 0.066	0.6244
Heart	0.588 ± 0.035	0.551 ± 0.026	0.4098
Spleen	0.099 ± 0.005	0.094 ± 0.010	0.6682
Bursa of Fabricius	0.266 ± 0.016	0.276 ± 0.043	0.8355
Pancreas	0.210 ± 0.016[a]	0.254 ± 0.010[b]	0.0438

Data are presented as means ± standard error of means.
Means within rows with different superscript alphabets are significantly different ([ab] $P < 0.05$).

The values of the monitored indicators of the fermentation process in the caecum are shown in Table 4. In the experimental group, compared to the control group, a significantly lower pH of the contents of the caecum was measured ($P < 0.05$). The addition of humic substances led to an increase in the content of the monitored short-chain fatty acids, but the difference compared to the control group was not statistically significant.

Table 4: Effect of humic substances on pH and concentration of short-chain fatty acids in the caecum content

	Control group	Experimental group	P-value
pH	6.93 ± 0.07[a]	6.62 ± 0.08[b]	0.0154
Acetic acid (mmol.L^{-1})	145.95 ± 8.31	151.42 ± 4.38	0.5714
Propionic acid (mmol.L^{-1})	27.22 ± 2.01	31.40 ± 3.03	0.2915
Butyric acid (mmol.L^{-1})	8.78 ± 0.97	8.93 ± 1.22	0.9267
Lactic acid (mmol.L^{-1})	29.18 ± 3.42	34.07 ± 3.97	0.3688

Data are presented as means ± standard error of means.
Means within rows with different superscript alphabets are significantly different ([ab] $P < 0.05$).

The content of dry matter and crude protein in chicken droppings are shown in Table 5. In the experimental group, a significantly higher content of dry matter ($P < 0.01$) and a significantly lower concentration of crude protein in dry matter of chicken droppings ($P < 0.05$) was found than in the control group.

Table 5: Effect of humic substances on content of dry matter and crude protein in droppings

	Control group	Experimental group	P-value
Dry matter ($g.kg^{-1}$)	166.9 ± 0.59^{a}	175.6 ± 2.15^{b}	0.0028
Crude protein ($g.kg^{-1}$ dry matter)	309.5 ± 3.36^{a}	301.1 ± 1.49^{c}	0.0459

Data are presented as means ± standard error of means.
Means within rows with different superscript alphabets are significantly different (^{ab} $P < 0.01$, ^{ac} $P < 0.05$).

4. DISCUSSION

The carcass yield of broilers as well as percentage share of abdominal fat pad in our experiment was not significantly affected by the addition of humic substances in the concentrations used. These results are in agreement with the results obtained in experiments on chickens by Ozturk et al. [17], Nagaraju et al. [18] and Arpášová et al. [19].

El-Husseiny et al. [5] reported opposite results in their experiment, where the carcass yield of chickens that received a feed mixture with the addition of humic substances in a concentration of 0.25 and 0.125 % was significantly higher and the proportion of abdominal fat pad was significantly lower than in the group without the addition of humic substances. Similar results were reported in the study by ELnaggar and El-Kelawy [20], who examined the effect of humic acids in Sasso chickens. Addition of humic acids to chicken diets in 0.1 and 0.2% concentration significantly increased the percentage of dressing and decreased percentage of abdominal fat compared to control group.

Improvement of carcass characteristics of chickens due to the influence of humic substances was also noted by Domínguez-Negrete et al. [21], Jaďuttová et al. [22] and A-Ghazalah et al. [23].

The results of various studies show that humic substances can have a positive effect on the carcass characteristics of chickens, but their effect depends on their origin, their physico-chemical properties, the method of application and the amount added to feed or water.

In the current study, we found a higher relative weight of the pancreas in the experimental group ($P < 0.05$). Abdel-Fattah et al. [24] reported that the enlargement of the pancreas indicates improved digestion and absorption of nutrients due to increased secretion and activity of pancreatic enzymes.

The relative weight of the other monitored visceral organs (liver, heart, spleen, bursa Fabricii) was not significantly affected by the addition of humic substances. Similar results were observed by Ozturk et al. [17], Nagaraju et al. [18] and Arif et al. [25]. Similarly, Rath et al. [26] reported no changes in the relative weights of heart, liver and spleen in broiler roosters receiving humic acid-enriched feed at 1.0 and 2.5 % concentration compared to the control group, but the weight of the bursa of Fabricius was significantly higher in the group with 2.5 % concentration, which indicates a positive immunostimulating effect of humic acids. ELnaggar and El-Kelawy [20] also noted the enlargement of the bursa of Fabricius due to humic acids.

The main product of microbial fermentation of non-starch polysaccharides is short-chain fatty acids, which are used by the host organism as an energy source, lead to a decrease in pH in the digestive tract, which can inhibit pathogenic bacteria and can accelerate the proliferation of intestinal epithelial cells. Shermer et al. [27] found that the addition of humate in amounts of 5 and 10 g.kg-1 of the diet had no significant effect on the concentration of short-chain fatty acids (acetic, propionic, isobutyric, butyric, isovaleric, valeric) in the contents of the caecum of broiler chickens.

These results are consistent with our findings. The concentration of individual fatty acids was not significantly affected by the addition of humic substances, but due to their higher content, there was a significant decrease in the pH of the caecum content compared to the control group ($P < 0.05$). These results show that the addition of humic substances leads to an increase in microbial fermentation in the cecum, which results in a decrease in the pH of the intestinal contents, which is unsuitable for the growth of pathogenic microorganisms [28].

The addition of humic substances led to a significant increase in the content of dry matter ($P < 0.01$) and a significant decrease in the content of crude protein ($P < 0.05$) in chicken droppings. The reduction of crude protein in chicken droppings may indicate a better utilization of nitrogenous substances and may lead to decreased production of volatile ammonia by microbial fermentation in the litter [12]. A higher dry matter content in droppings may improve the microclimate in the poultry house, too. The lower water content in the litter limits microbial fermentation [29].

5. CONCLUSION

The results of our study show that the supplementation of humic substances to diets of broiler chickens does not have a significant effect on carcass characteristics, but can lead to increase in microbial fermentation in the caecum of chickens, which was manifested by non-significantly higher concentration of short-chain fatty acids and by a significant decrease in the pH of the contents of the caecum. The significant increase in the dry matter content and the significant decrease in the content of crude protein in the dry matter of chicken droppings indicate that humic substances can contribute to reducing the burden on the environment from poultry farms.

6. CONFLICTS OF INTERESTS

Authors declare that there are no conflicts of interest.

7. ACKNOWLEDGEMENT

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